D-aminoacyl esters of maytansinol. This material can be further purified using a Diazem™ cyano preparative HPLC column (250 mm×50 mm, 10 micron particle size) equilibrated in a mixture of hexanes:2-propanol:ethyl acetate (17:2:6, v/v/v) at a flow rate of 150 mL/min as described above for **10*b***.

Esterification of Maytansinol (**2**) with N-methyl-N-(phenyldithio-propanoyl)-L-alanine (**6*e***)

A solution of N-methyl-N-(phenyldithio-propanoyl)-L-alanine (**6*e***) (31.5 mg, 105 μmol) in methylene chloride (0.4 mL) was stirred under argon and treated sequentially with solutions of DCC (26 mg, 126 μmol) in methylene chloride, 1M $ZnCl_2$ (17.7 μmol) in ether and maytansinol (**2**) (10 mg, 17.7 μmol) in methylene chloride (0.2 mL). The reaction mixture was stirred at room temperature for three hours. The precipitate was removed by filtration and the filtrate concentrated under reduced pressure.

The mixture can be further purified using a Diazem™ cyano preparative HPLC column (250 mm×50 mm, 10 micron particle size) was equilibrated in a mixture of hexanes:2-propanol:ethyl acetate (17:2:6, v/v/v) at a flow rate of 150 mL/min as described above for (**10*b***).

^{1}H NMR ($CDCl_3$) L-aminoacyl isomer (**10*e***): δ0.82 (3H, s), 1.11–1.25 (1H, m), 1.33 (3H, d, J=3 Hz), 1.61 (3H, s), 1.63 (3H, d, J=14 Hz), 2.19 (1H, dd, J=13 Hz and 15 Hz), 2.61 (1H, dd, J=12 Hz and 15 Hz), 2.78 (3H, s), 2.68–3.03 (2H, m), 3.07 (1H, d, J=9 Hz), 3.20 (3H, s), 3.38 (3H, s), 3.53 (1H, d, J=9 Hz), 3.63 (1H, d, J=13 Hz), 3.68 (3H, s), 4.01 (3H, s), 4.30 (1H, t, J=11Hz), 4.79 (1H, dd, J=3 Hz and 8 Hz), 5.43 (1H, q, J=7 Hz), 5.68 (1H, dd, J=9 Hz and 15 Hz), 6.23 (1H, s), 6.45 (1H, dd, J=12 Hz and 15 Hz), 6.60 (1H, d, J=1.5 Hz), 6.75 (1H, d, J=12 Hz), 6.77 (1H, d, J=1.5 Hz), 7.22–7.40 (5H, m).

Esterification of Maytansinol (**2**) with N-methyl-N-(3-methyldithio-butanoyl)-L-alanine (**6*g***)

A solution of N-methyl-N-(3-methyldithiobutanoyl)-L-alanine (**6*g***) (23.2 mg, 0.088 mmol) in dichloromethane (0.2 mL) was stirred under an argon atmosphere, and treated sequentially with a solution of maytansinol (5 mg, 0.0088 mmol) in dichloromethane (0.2 mL), DCC (20.6 mg) in dichloromethane (0.2 mL) and 1M zinc chloride (0.0088 mmol) in ether. The reaction was stirred overnight at room temperature. The reaction mixture was then filtered and the solvent was evaporated. The residue was purified by preparative TLC on silica gel, using 6% methanol in chloroform to give the desired L-maytansinoid ester **10*g***. The product **10*g*** can be more efficiently purified using a_Diazem™ cyano preparative HPLC column (250 mm×10 mm, 10 micron particle size) equilibrated in a mixture of hexanes:2-propanol:ethyl acetate (17:2:6, v/v/v) at a flow rate of 4.70 mL/min as described above for (**10*b***).

Esterification of Maytansinol (**2**) with N-acetyl-N-methyl methyldithiocysteine (**9*a***)

A solution of N-Acetyl-N-methyl-methyldithiocysteine (**9*a***) (15.6 mg, 0.07 mmol) in dry methylene chloride (0.45 mL) was stirred at room temperature under an argon atmosphere and treated sequentially with solutions of 1M $ZnCl_2$ in ethyl ether (0.028 mmol), DCC (17.3 mg, 0.084 mmol) in methylene chloride (0.2 mL), and maytansinol (**2**) (4.0 mg, 0.007 mmol) in methylene chloride (0.1 mL). The reaction mixture was stirred for three hours and then filtered and the filtrate evaporated under reduced pressure.

The residue can be further purified using a Diazem cyano preparative HPLC column (250 mm×50 mm, 10 micron particle size) was equilibrated in a mixture of hexanes:2-propanol:ethyl acetate (17:2:6, v/v/v) at a flow rate of 150 mL/min as described above for (**10*b***).

Step 3

Reduction of Disulfide-Containing Maytansinoid

A solution of the disulfide-containing L-aminoacyl ester of maytansinol **10*b*** (1.95 g, 2.5 mmol) in a mixture of ethyl acetate (140 mL) and methanol (210 mL) was stirred at room temperature under an argon atmosphere, and treated with a solution of dithiothreitol (0.95 g, 6.2 mmol) in 0.05 M potassium phosphate buffer (140 mL), pH 7.5, containing 2 mM ethylenediaminetetraacetic acid (EDTA). The progress of the reaction was monitored by HPLC and was complete in three hours.

The completed reaction mixture was treated with a solution of 0.2 M potassium phosphate buffer (250 mL), pH 6.0, containing 2 mM EDTA, and then extracted with ethyl acetate (3×600 mL). The organic layers were combined, washed with brine (100 mL) and then dried over sodium sulfate. Evaporation of the solvent gave a residue of crude thiol-containing maytansinoid **11*b***.

The crude thiol-containing maytansinoid **11*b*** was purified by HPLC using a preparative Diazem™ cyano HPLC column (250 mm×50 mm, 10 micron particle size) that was equilibrated in a mixture of hexanes:2-propanol:ethyl acetate (78.0:5.5:16.5, v/v/v) and ran at a flow rate of 150 mL/min. Thiol-containing maytansinoid **11*b*** eluted as peak centered at 16 min. Fractions containing the product were evaporated to give pure thiol-containing maytansinoid **11*b*** as a white solid (76% yield with a purity of 99%).

The presence of one mole of sulfhydryl group/mol product was confirmed using Ellman's assay. The product was further characterized by NMR spectroscopy. ^{1}H NMR ($CDCl_3$): δ0.84 (3H, s), 1.33 (3H, d, J=5 Hz), 1.35 (3H, d, J=5 Hz), 1.60 (3H, s), 1.68 (3H, s), 2.22 (1H, dd, J=3 Hz and 14 Hz), 2.60–2.82 (2H, m), 2.88 (3H, s), 3.08–3.20 (2H, m), 3.25 (3H, s), 3.39 (3H, s), 3.55 (1H, d, J=9 Hz), 3.71 (1H, d, J=12 Hz), 4.02 (3H, s), 4.32 (1H, t, J=10 Hz), 4.81 (1H, dd, J=3 Hz and 12 Hz), 5.45 (1H, q, J=7 Hz), 5.67 (1H, dd J=9 Hz and 15 Hz), 6.25 (1H, s), 6.47 (1H, dd, J=11 Hz and 15 Hz), 6.70 (1H, d, J=1.5 Hz), 6.75 (1H, d, J=11 Hz), 6.86 (1H, d, J=1.5 Hz).

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

What is claimed is:

1. A process for preparing a thiol-containing maytansinoid wherein the thiol group is part of the ester moiety at C-3, comprising the steps of:

(1) conducting reductive hydrolysis of a maytansinoid C-3 ester with a reducing agent selected from the group consisting of lithium trimethoxyaluminum hydride ($LiAl(OMe)_3H$), lithium triethoxyaluminum hydride ($LiAl(OEt)_3H$) and lithium tripropoxyaluminum hydride ($LiAl(OPr)_3H$), to yield a maytansinol;

(2) purifying the maytansinol to remove side products when present;

(3) esterifying the purified maytansinol with a carboxylic acid to yield a reaction mixture of an L- and a D-aminoacyl ester of maytansinol;

(4) separating the L-aminoacyl ester of maytansinol from the reaction mixture in (3);

(5) reducing the L-aminoacyl ester of maytansinol to yield a thiol-containing maytansinoid; and

(6) purifying the thiol-containing maytansinoid.

2. The process of claim **1**, wherein the reducing agent in (1) is lithium trimethoxyaluminum hydride.

3. The process of claim 1, wherein the reducing agent in (1) is used in a concentration of from about 5 to 100 equivalents per mole of the maytansinoid C-3 ester.

4. The process of claim 1, wherein the reducing agent in (1) is used in a concentration of from about 7.5 to 30 equivalents per mole of the maytansinoid C-3 ester.

5. The process of claim 1, wherein the reducing agent in (1) is used in a concentration of from about 10 to 20 equivalents per mole of the maytansinoid C-3 ester.

6. The process of claim 1, wherein the reductive hydrolysis in (1) is conducted at a temperature of from about −80° C. to 0° C.

7. The process of claim 1, wherein the reductive hydrolysis in (1) is conducted at a temperature of from about −45° C. to −27.5° C.

8. The process of claim 1, wherein the reductive hydrolysis in (1) is conducted at a temperature of from about −35° C. to −30° C.

9. The process of claim 1, wherein the reducing agent in (1) is added over a period of from about 5 to 40 minutes.

10. The process of claim 1, wherein the reducing agent in (1) is added over a period of from about 7 to 20 minutes.

11. The process of claim 1, wherein the reducing agent in (1) is added over a period of from about 8 to 12 minutes.

12. The process of claim 1, wherein the maytansinol is purified in (2) by chromatography.

13. The process of claim 12, wherein the chromatography is silica gel column chromatography, preparative thin-layer chromatography on silica gel or cyano-bonded silica HPLC column chromatography.

14. The process of claim 12, wherein the chromatography is silica gel column chromatography.

15. The process of claim 12, wherein the purification is performed at ambient temperature.

16. The process of claim 12, wherein the maytansinol is purified to a purity of about 95%.

17. The process of claim 1, wherein the carboxylic acid in (3) is selected from the group consisting of N-methyl-N-methyldithioacetyl-L-alanine, N-methyl-N-(3-methyldithio-propanoyl)-L-alanine, N-methyl-N-(3-methyldithio-butanoyl)-L-alanine, N-methyl-N-(4-methyldithio-butanoyl)-L-alanine, N-methyl-N-(5-methyldithio-pentanoyl)-L-alanine, N-methyl-N-(3-phenyldithio-propanoyl)-L-alanine, N-methyl-N-(3-(4-nitrophenyldithio) propanoyl)L-alanine, N-acetyl-N-methyl-methyldithiocysteine and N-acetyl-N-methyl-methyldithiohomocysteine.

18. The process of claim 1, wherein the carboxylic acid in (3) is N-methyl-N-(3-methyldithio-propanoyl)-L-alanine.

19. The process of claim 1, wherein the esterification in (3) is conducted at ambient temperature.

20. The process of claim 1, wherein the esterification in (3) further comprises the use of dicyclohexylcarbodiimide and zinc chloride.

21. The process of claim 1, wherein the separating in (4) is carried out by passing the reaction mixture over a cyano-bonded silica HPLC column.

22. The process of claim 1, wherein the separating in (4) is carried out at about 25° C.

23. The process of claim 1, wherein the reduction in (5) uses dithiothreitol as the reducing agent.

24. The process of claim 1, wherein the reduction in (5) is carried out in a mixture of ethyl acetate-methanol-aqueous buffer which is capable of keeping buffer salts, dithiothreitol, unreduced maytansinoids and reduced maytansinoids in solution.

25. The process of claim 24, wherein the mixture of ethyl acetate-methanol-aqueous buffer is 1:1.5:1, v/v/v, ethyl acetate:methanol:aqueous buffer.

26. The process of claim 24, wherein the concentration of the thiol-containing maytansinoid is such that the thiol-containing maytansinoid remains soluble in ethyl acetate-methanol-aqueous buffer.

27. The process of claim 26, wherein the concentration of the thiol-containing maytansinoid is about 4 g/L.

28. The process of claim 1, wherein the reduction in (5) is carried out in an oxygen-free atmosphere.

29. The process of claim 1, wherein the reduction in (5) is carried out at about 25° C.

30. The process of claim 1, wherein the purifying of the thiol-containing maytansinoid in (6) is by chromatography.

31. The process of claim 30, wherein the purifying of the thiol-containing maytansinoid in (6) is by a cyano-bonded HPLC column chromatography.

32. The process of claim 31, wherein the chromatography is by a cyano-bonded HPLC column equilibrated and run in an organic solvent.

33. The process of claim 32, wherein the organic solvent is a mixture of hexanes:2-propanol:ethyl acetate.

34. The process of claim 33, wherein the organic solvent is a 78.0:5.5:16.5, v/v/v, mixture of hexanes:2-propanol:ethyl acetate.

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